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Histochemical and morphometric characteristics of the normal human vastus medialis longus and vastus medialis obliquus muscles

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ABSTRACT

The histochemical and morphometric characteristics of the vastus medialis longus and vastus medialis obliquus muscles were studied and compared with data on vastus lateralis. Cross-sections of autopsied muscles from 9 healthy men, aged 18–44 y, who had died suddenly were analysed. Data were obtained on proportions, cross-sectional diameter, and on atrophy and hypertrophy factors, of type 1, 2a, 2b, and 2c fibres. The analysis showed that the anatomical differences and the different functional demands placed on vastus medialis longus and vastus medialis obliquus are also expressed in different proportions and sizes of fibre types in the two muscles. The proportion of type 1 fibres was significantly higher ($P < 0.01$), and the proportion of 2b fibres was significantly lower ($P < 0.01$) in vastus medialis longus than in vastus medialis obliquus. The diameters of type 1 and type 2a fibres were significantly smaller ($P < 0.01$) in vastus medialis longus than in vastus medialis obliquus, although the differences were small. Within muscles a nonrandom arrangement of fibre types existed with the deeper portions of the muscles having more type 1 fibres than the more superficial portions. The histochemical and morphometric characteristics of vastus lateralis and vastus medialis obliquus show great similarity, reflecting the common function of both muscles which is taking part in transverse knee stability. Estimates of the limits of normality of the proportion, diameter, atrophy and hypertrophy factors of type 1, 2a, 2b, and 2c fibres might be useful in obtaining information on how different physiological and pathological conditions influence the proportion and size of different fibre types. This information could also be helpful in planning of specific muscle-training programmes with the purpose of restoring normal muscle function.

Key words: Skeletal muscle; quadriceps femoris; fibre type analysis.

INTRODUCTION

Quadriceps femoris is a powerful extensor of the knee joint. When the knee is hyperextended quadriceps is not required for maintenance of the erect position, but when flexion is initiated, quadriceps is strongly activated so as to prevent a fall resulting from knee flexion. Quadriceps consists of 4 muscles: vastus intermedius (VI), vastus lateralis (VL), and vastus medialis (VM) are monoarticular muscles, and rectus femoris (RF) is biarticular. For many years it was thought that vastus medialis primarily functioned in the last 15° of knee extension. However, detailed studies have repeatedly failed to support this belief, indicating that all 4 muscles of the quadriceps are active early and throughout the range of motion. A thorough account of the vastus medialis controversy can be found in Speakman & Weisberg (1977).

A close inspection of vastus medialis demonstrates that muscle fibres follow two distinctly different alignments, which are also anatomically distinct. The two parts of VM can be differentiated by means of a femoral nerve branch, which runs superficially, in the areolar fascial plane, or deeply between them (Weinstabl et al. 1989). A division of vastus medialis into long and oblique components was introduced by Lieb & Perry (1968). Vastus medialis longus (or pars longus) (VML), the proximal part of vastus medialis,

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inserts at the base of the patella. The fibres in this long head are directed vertically, deviating an average of 15°–18° medially from the longitudinal axis of the femur. Vastus medialis obliquus (or pars obliquus) (VMO), the distal part of vastus medialis, mainly originates from the adductor magnus tendon and inserts at the medial margin of the patella (Bose & Kanagasuntheram, 1980). The fibres in this short head are more horizontal, deviating an average of 50°–55° medially from the femoral axis. (Fig. 1). Disuse of extensor muscles, secondary to knee joint trauma, arthritis, and infection, often results in atrophy which, based on clinical observations, predominantly affects the distal part of VM. Vastus medialis longus and vastus medialis obliquus are also functionally different (Lieb & Perry, 1968; Speakman & Weisberg, 1977; Bose & Kanagasuntheram, 1980; Basmajian & DeLuca, 1985). Vastus medialis longus is purely an extensor of the leg. Vastus medialis obliquus is a weak extensor of the leg, but it plays an important role in keeping the patella on track in gliding on the femoral condyles. The medially directed forces of the VMO counteract laterally directed forces of the vastus lateralis, thus preventing lateral displacement of the patella in the trochlear groove. A biomechanical imbalance of the two muscles may lead to abnormal patellar tracking, which may elevate patellofemoral contact pressures, causing pain and participate to pathological changes in patellofemoral articular cartilage (Fox, 1975; Outerbridge & Dunlop, 1975; Insall, 1982).

Histochemical and morphometric analyses are among the most useful ways to analyse the structure and function of healthy and diseased human skeletal muscle (Johnson et al. 1973; Polgar et al. 1973; Bennington & Krupp, 1984; Dubowitz, 1985; Engel, 1986), which is characteristically composed of fibres with different structural and functional properties. Based on the alkaline and acidic lability of the myofibrillar adenosine triphosphatase (mATPase) activity, the fibres can be classified into different fibre types (Brooke & Kaiser 1970; Dubowitz, 1985). At alkaline pH, the type 1 (slow-twitch) fibres have a low mATPase activity, whereas type 2 (fast-twitch) fibres react strongly. A further subdivision of type 2 fibres into types 2a, 2b, and 2c fibres can be achieved by preincubation at different levels of acidity. Histochemical and morphometric characteristics of normal muscle fibre types depend on age (Essén-Gustavsson & Borges 1986; Lexell et al. 1988; Oertel, 1988; Glenmark et al. 1992), sex (Essén-Gustavsson & Borges, 1986; Glenmark et al. 1992) and the specific muscle studied (Johnson et al. 1973; Polgar et al. 1973). In normal muscles there is also a considerable interindividual and intersample variation regarding fibre type proportion and size (Sandstedt, 1981; Nygaard & Sanchez, 1982; Mahon et al. 1984; Eržen et al. 1990). However, it is not clear whether the distribution of fibre types varies systematically as a function of depth. Studies on human vastus lateralis muscles gave conflicting results (Nygaard & Sanchez, 1982; Lexell et al. 1983; Eržen et al. 1990). The size also varies from fibre to fibre in the same sample. Exercise (Gollnick et al. 1972; Costill et al. 1979), inactivity (Lindboe & Platou, 1982), immobilisation (Booth & Kelso, 1973), electrical stimulation (Pette & Vrbova, 1992) and different muscle diseases (Brooke & Engel, 1969; Verma et al. 1992) can change the normal values of proportion and size. An obvious prerequisite in the study of pathological or physiological conditions, influencing the histochemical and morphometric characteristics of different fibre types, is to determine the best possible estimate of the limits of normality in healthy individuals comparable by age, sex and muscle. Because many characteristics of muscle fibres are influenced by the pattern of usage (Saltin et al. 1977), it was hypothesised that the VMO and VL muscles would show a high degree of histochemical and morphometric similarity, and that VML would differ both from VMO and VL. The purpose of this study was thus to investigate the histochemical and morphometric characteristics of VML and VMO in young healthy males in order to establish the normal limits of these characteristics, to make comparisons with VL muscles, and to study the fibre type distributions between superficial and deep sites.

MATERIALS AND METHODS

Sections of the VML and VMO muscles were obtained at autopsy from 9 young men (mean age 29.3 y, range 18–44 y) who had suffered sudden death. None of the subjects had a history of neuromuscular disease, and postmortem examinations revealed no evidence of pathological abnormalities. All specimens were obtained from the right leg within 15 h after death.

Preparative procedure

Two slices, about 10 mm thick, were cut from the whole vastus medialis muscle. The first slice was cut from the VML approximately 15 cm proximal to the base of the patella and the second from the VMO near the superomedial border of the patella. Three blocks, each measuring approximately 1 cm^3, from the super-

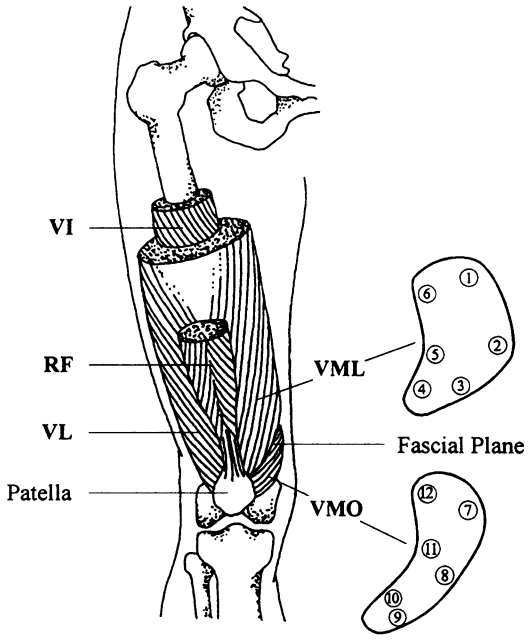

Fig. 1. Separation of vastus medialis longus (VML) and vastus medialis obliquus (VMO) muscles, and sites of sampling indicated on transverse sections of VML (1–3 superficial region, 4–6 deep region) and VMO (7–9 superficial region, 10–12 deep region).

ficial and 3 from the deep portion of each muscle (Fig. 1) were cut from the slices and frozen in liquid nitrogen. Myofibrillar adenosine triphosphatase (mATPase) activity was demonstrated in serial 10 μm transverse sections with the calcium method (Padykula & Herman, 1955) at pH 9.4, and after preincubation at pH 4.6 and 4.3 (Guth & Samaha, 1969). The sections stained for mATPase activity in alkaline medium were fixed in 2% paraformaldehyde in 0.1 N cacodylate buffer pH 7.2 with addition of 0.4 M sucrose. In each section a fascicle, not comprising smaller fascicles and well defined by its perimysium, was photographed by an Opton photomicroscope with an average total linear magnification of ×115. By combining observations from 3 serial cross-sections, the muscle fibres were classified as type 1, 2a, 2b and 2c fibres (Brooke & Kaiser, 1970).

Data acquisition and preprocessing

Photographs of the transverse sections stained for mATPase activity in alkaline medium were used to measure the size of fibres. The contours of all fibres within a selected fascicle were digitised with the aid of a Cherry graphic tablet connected to an IBM PC/AT compatible computer which also served for further calculations. From the fibre boundary points, the lesser diameter was calculated by treating each fibre in a cross-section as an ellipse. Individual fibre diameters were used to determine means, standard deviations, and to obtain the lower (*ll*) and upper (*ul*) limits of normal diameters of different fibre types. The atrophy (*AF*) and hypertrophy (*HF*) factors, and the upper limits of these factors (*ul*(*AF*) and *ul*(*HF*)) accepted as being normal, were determined from the diameters as described by Pernuš & Eržen (1994). Briefly, the lower limits, *ll*, were set at the 2.5 centile and the upper limits, *ul*, at the 97.5 centile on the fibre type diameter density curve. The atrophy and hypertrophy factors, which express the number and extent of abnormally small and abnormally large type *t*, $t = 1$, 2a, 2b, 2c fibres, were defined as

$$AF(t) = \frac{1000}{N(t)} \sum_{\substack{i=1 \\ d_i(t) < ll(t)}}^{N(t)} w(d_i(t))$$

$$HF(t) = \frac{1000}{N(t)} \sum_{\substack{i=1 \\ d_i(t) > ul(t)}}^{N(t)} w(d_i(t))$$

where $N(t)$ is the number of type t fibres in a sample, d the diameter of a fibre, and w the weight, given to a fibre diameter in linear proportion to the distance for which the diameter falls outside the normal range. The upper limits of atrophy $ul(AF(t))$ and hypertrophy $ul(HF(t))$ factors accepted as being normal were set at the 95th centile on the atrophy or hypertrophy density curve.

Statistical methods

Throughout the data analysis, the *SYSTAT* (version 5.03) statistical package (SYSTAT, Inc.) was used to calculate means and standard deviations (S.D.) as well as for simultaneous pairwise comparison (Bonferroni procedure).

RESULTS

For each individual subject, data on the age, number of fibres analysed, and the fibre type proportions are given in Table 1 (VML) and Table 2 (VMO). In both muscles, the S.D.s and ranges indicate a considerable intersample variation in fibre type proportions. In VML, the mean differences between the highest and lowest intrasubject proportions of type 1, 2a, and 2b fibres were 24, 24.5 and 13.5%, respectively, and in VMO these mean differences were 27, 21.7 and 21.9%, respectively. Interindividual differences were also apparent. In VML the mean proportion of type 1 fibres was higher than the mean proportion of type 2a and 2b fibres in all subjects except subject 5, whereas in VMO this was true in 6 subjects. In all subjects the proportion of type 1 fibres was higher in VML than in VMO. In 6 subjects the difference was significant ($P <$

Table 1. *Fibre type proportions within the fascicles of vastus medialis longus*

Subject	Age (y)	No. of fibres	Proportion of type 1 fibres			Proportion of type 2a fibres			Proportion of type 2b fibres		
			%	S.D.	Range	%	S.D.	Range	%	S.D.	Range
1	18	855	56[a]	9	42–66	31	13	8–45	10[b]	9	0–24
2	18	688	65[a]	7	55–72	35[a]	7	28–45	0	0	0–0
3	22	515	51	10	36–61	28	12	12–48	15	12	2–26
4	23	647	65[a]	10	49–77	28[b]	7	18–37	4	6	0–14
5	26	538	39[a]	8	30–52	55	10	41–67	5[a]	4	0–10
6	31	858	59	10	43–68	29	5	20–32	12	9	0–24
7	40	841	68[a]	11	53–79	24	9	11–38	8[b]	6	0–15
8	42	506	71[b]	6	64–82	28	6	18–36	1[a]	1	0–3
9	44	895	62	12	44–75	35	12	24–53	3[a]	4	0–8

[a] Significantly different ($P < 0.01$) from VMO (Table 2). [b] Significantly different ($P < 0.05$) from VMO (Table 2).

Table 2. *Fibre type proportions within the fascicles of vastus medialis obliquus*

Subject	Age (y)	No. of fibres	Proportion of type 1 fibres			Proportion of type 2a fibres			Proportion of type 2b fibres		
			%	S.D.	Range	%	S.D.	Range	%	S.D.	Range
1	18	1019	35	5	26–41	36	7	25–47	25	10	13–37
2	18	679	43	9	35–58	55	8	42–64	0	0	0–0
3	22	667	42	8	30–51	28	7	20–37	19	12	5–34
4	23	680	41	15	25–63	40	7	33–51	4	4	1–12
5	26	443	26	6	17–33	49	10	30–59	21	12	1–37
6	31	865	51	10	38–66	31	10	18–41	17	10	2–28
7	40	653	46	12	29–66	35	8	25–47	18	8	9–29
8	42	739	58	11	42–75	31	10	20–43	11	8	2–22
9	44	769	53	10	41–65	26	9	16–36	19	12	0–31

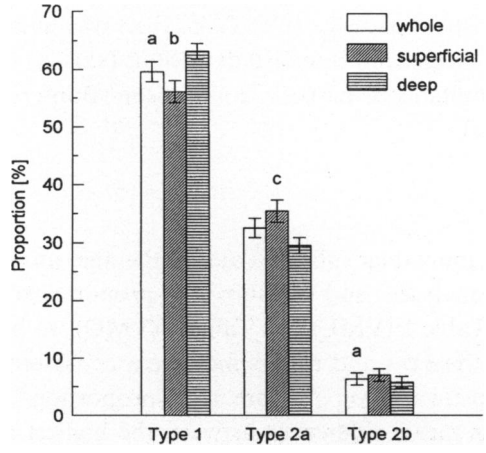

Fig. 2. Fibre type composition of whole and of superficial, and deep portions of VML. Bars denote means and the vertical lines ±S.E.M. (a) Significantly ($P < 0.01$) different from VMO (Fig. 3). (b, c) Significant differences ($P < 0.01$ and $P < 0.05$, respectively) between superficial and deep portions.

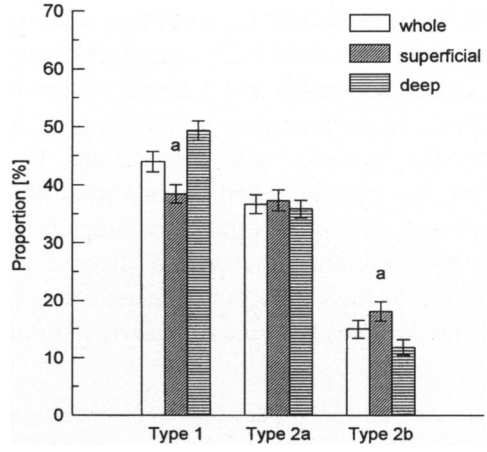

Fig. 3. Fibre type composition of whole and of superficial, and deep portions of VMO. Bars denote means and the vertical lines ±S.E.M. (a) Significant differences ($P < 0.01$) between superficial and deep portions.

0.05). The proportion of 2b fibres was higher in VMO than in VML in 6 subjects and the difference was significant ($P < 0.05$) in 5 subjects. In all samples of the VMO, except in samples of subject 2 and 1 sample of subject 9, the proportion of 2b fibres differed from 0. In VML there were no type 2b fibres in 19 out of 54 samples.

The fibre type composition obtained from the data for all samples and from samples from the superficial (S) and deep (D) sites of VML and VMO, are shown in Figures 2 and 3. The proportion of type 1 fibres was

Table 3. *Mean, S.D., lower and upper limit of diameters, and upper limits of atrophy and hypertrophy factors of VML and VMO muscles.*

Muscle		Muscle fibre type 1	2a	2b
VML	Mean	60.1[a]	59.2[a]	56.5
	S.D.	12.5	12.2	12.3
	ll	36.9	35.9	34.2
	ul	85.9	82.8	84.2
	ul(AF)	41	26	20
	ul(HF)	89	24	20
VMO	Mean	63.8	63.9	56.7
	S.D.	13.5	14.4	14.2
	ll	39.1	38.4	31.9
	ul	91.4	94.9	85.6
	ul(AF)	64	55	62
	ul(HF)	75	57	60

[a] Significantly different ($P < 0.01$) from VMO.

significantly ($P < 0.01$) higher and the proportion of 2b fibres was significantly ($P < 0.01$) lower in VML than in VMO. The proportion of 2a fibres did not differ significantly between the 2 muscles. Systematic differences were found between sites. In both muscles the proportion of type 1 fibres was higher in samples taken from the deep portion than in those from the superficial part. The difference was statistically significant ($P < 0.01$). The opposite was true for the proportions of type 2a and 2b fibres.

Table 3 gives the mean, S.D., lower, and upper limit of the diameters, and the upper limits of fibre type atrophy and hypertrophy factors in all samples of VML and VMO, respectively. In both muscles high S.D.s reflect the intrasample and intersample differences, as well as a variation between subjects. Because of this large variability the ranges of normal fibre type diameters determined as *ul-ll* were high. In VML the highest intersubject differences (not given here) of type 1, 2a, and 2b, mean diameters were 16.5, 18.3, and 21.7 μm, respectively. In VMO these differences were 17.0, 14.5, and 27.3 μm, respectively. The mean fibre type sizes were within the size ranges of each subject. In both muscles type 2b fibres were the smallest and type 2a fibres the largest in most subjects. The diameters of all fibre types, except type 2b fibres, differed significantly ($P < 0.01$) between the 2 muscles. Fibres of all types were larger in VMO than in VML. Although the differences between the mean diameters were significant, they were small. Type 1 fibres were larger in VMO than in VML in 8 subjects and type 2a fibres in 7 subjects.

DISCUSSION

Data on the range of normal values of different histochemical and morphological features in different human muscles, in both sexes and in different age groups, are essential for the understanding of a normal muscle, as well as in the study of pathological and/or physiological conditions, influencing the histochemical and morphological features of different fibre types. Quantitative values may also be of use when designing treatment protocols, which incorporate exercises to strengthen a muscle or a group of muscles selectively. The sampling error, which may occur due to the small size of specimens, is well known (Sandstedt, 1981; Nygaard & Sanchez, 1982; Eržen et al. 1990). Therefore, to set the standard values for mean, lower and upper limits of the proportions and sizes of different fibre types, and for the upper limits of the atrophy and hypertrophy factors, a representative number of subjects, samples, and fibres have to be analysed. The reasons for this are the intersubject, intersample, and intrasample histochemical and size differences. For ethical and practical reasons, it is difficult to obtain large numbers of biopsies from normal individuals, therefore data from autopsy material are used to provide normal values. In the present study, in which samples were obtained from the right leg, the preferential usage of lower limbs was unknown. Since several studies (Blomstrand et al. 1984; Essén-Gustavsson & Borges, 1986; Eržen et al. 1990) indicated that there were no significant differences in muscle fibre type proportion and size between the right and left leg, we believe that standard values, obtained in this study, could be representative for both legs.

The apparent intersample and interindividual variability in fibre type composition and size, as obtained in the present study, is in accordance with observations on other human muscles (Sandstedt, 1981; Nygaard & Sanchez, 1982; Lexell et al. 1983; Lindman et al. 1990). It is well known that the skeletal muscle, due to its extreme plasticity, is able to adapt quickly to changed physiological requirements, and to respond to experimental conditions (Pette, 1980). Because of the marked intersample variability of histochemical and size characteristics, it would also be possible to use the standard data as a reference in various studies especially when pathological and/or physiological conditions influence only one limb (immobilisation, exercise, injuries of the anterior cruciate ligament, meniscus rupture, cartilage damage, and arthritis). Thus taking biopsy specimens from the other ('normal') extremity, as an adequate control

material, could be avoided. Because disuse of one lower limb affects the normal kinematics of the whole body, it is questionable whether the biopsy taken from the unaffected leg is any better as reference material as is the standard data.

The present study shows that the anatomical differences and the different functional demands, placed on the VML and VMO, are also expressed in different proportions and sizes of muscle fibre types in the two muscles. Vastus medialis longus and vastus medialis obliquus significantly ($P < 0.01$) differ in the proportion of type 1 (59.6%:44%) and type 2b (6.3%:15%) fibres. The VML muscle is almost entirely composed of type 1 and type 2a fibres. In many samples of this muscle no 2b fibres were found. The proportion of slow-twitch type 1 fibres is nearly twice as high as the proportion of fast-twitch type 2a fibres. In VMO, the proportion of fast-twitch type 2 fibres is higher than the proportion of type 1 fibres. These observations indicate that VML is a slower and more fatigue-resistant muscle than VMO. These characteristics correspond to the different functions of the VML, which is an extensor of the knee and to the VMO, which maintains the stable position of the patella in the femoral groove. The present data do not support the hypothesis that, besides proportions, differences in fibre type diameters could be an indication of different functions of the VML and VMO. Although the diameters of type 1 and 2a fibres were significantly smaller ($P < 0.01$) in VML than in VMO, type 1 fibres were on the average only 3.7 μm smaller and type 2a fibres only 4.7 μm smaller in VML than in VMO. We are not aware of any publications on the proportions and diameters of type 1, 2a, 2b and 2c fibres in VML and VMO, although Lindboe & Platou (1982) reported on the proportions and cross-sectional areas, Johnson et al. (1973) on the proportions, and Polgar et al. (1973) on the diameters of type 1 and 2 fibres in vastus medialis.

Recently, we have analysed the vastus lateralis (VL) muscle of young, 18–29-y-old healthy men (Pernuš & Eržen, 1994). Although the subjects, analysed in both studies, were not the same we believe that, because the same preparative procedure and the same methods were used in the present and previous investigations, the 3 muscles may be compared with each other. Simultaneous pairwise comparisons of the proportion of different fibre types in the 3 muscles showed that VL and VML differ significantly ($P < 0.01$) in the proportion of type 1 fibres, whereas the proportion of this type of fibre was not significantly different between VL and VMO. The proportion of type 2a fibres was not significantly different between the three muscles, whereas all muscles significantly differed between themselves as type 2b fibres are concerned. The vastus lateralis muscle has the highest proportion of 2b fibres. Grabiner et al. (1991) conducted an investigation to examine effects of fatiguing, isometric and dynamic knee extension exercise on the relative fatigability of the VMO and VL in a group of subjects with no knee pathology. His results showed statistically similar fatigability for the VMO and VL with an indication of a trend in the dynamic experiment, suggesting a greater propensity for fatigue in the VL. The present finding, that the proportion of 2b fibres is higher in VL than in VMO, is in agreement with these observations. Fibre type composition directly affects fatigability. Fast-twitch glycolytic type 2b muscle fibres, which produce greater amounts of lactic acid as a normal by-product of anaerobic metabolism than do slow-twitch oxidative type 1 fibres and fast-twitch oxidative-glycolytic type 2a fibres, are less fatigue resistant than type 1 and 2a fibres. With respect to the diameters of type 1, 2a, 2b, and 2c fibres VL differed significantly from both VML and VMO. The differences, however, were small. The largest difference (7.9 μm) was found between the diameter of type 1 fibres in VL and VMO. It seems that the fibres in VL are somewhat smaller than the fibres in VML and VMO. The present findings are in agreement with the observation made by Polgar et al. (1973) that 'the main way in which human muscles are adapted to fulfill their varying physiological roles is not by means of any constant or conspicuous difference in the sizes of the different fibre types, but rather by means of their varying numerical fibre type constitution'.

Changes in the fibre size, which may have a physiological and/or pathological basis, can lead to fibre type atrophy or hypertrophy, which can be expressed by atrophy and hypertrophy factors (Brooke & Engel, 1969; Pernuš & Eržen, 1994). These factors reflect the number and extent of abnormally small and abnormally large fibres, i.e. fibres whose size falls outside the range determined by the lower (*ll*) and upper (*ul*) limits of normal fibre type diameters. By calculating the fibre type AF and HF in a sample, and by comparing these factors with the accepted upper limits of normal, the extent of atrophy or hypertrophy can be determined objectively and quantitatively. Moderate restriction of movement of some weeks' duration leads to a clear atrophy of an affected muscle. Different causes of disturbance of motility, such as immobilisation, patellofemoral pain, instability of the knee joint, and arthritis, may affect the size and proportion of fibre types differently. Establishing a connection between the cause of

disturbance of motility and both fibre type proportion and atrophy might provide some useful information for planning of specific muscle-training programmes for restoring normal muscle function. Atrophy of VM was studied in patients with chronic instability of the anterior cruciate ligament (Edström, 1970; Baugher et al. 1980) and meniscus rupture (Lindboe & Platou, 1982). The histochemical studies of biopsy specimens yielded contradictory results. Edström (1970) found selective atrophy of varying degree in type 1 fibres. Although the exact location of the biopsy was not indicated, it can be concluded that, since biopsies were performed in conjunction with arthrotomy, they were taken from VMO. Baugher et al. (1980), however, reported a selective decrease of type 2 fibre size, and recommended a specific programme of strength and power exercises for patients with deficiency of the anterior cruciate ligament. Lindboe & Platou (1982), who studied patients with the rupture of a meniscus, found that the type 1 and type 2 fibres were equally affected with a size reduction of 27.6 and 25%, respectively.

The present study shows that the fibre type distribution of VML and VMO is not random. There is a systematic difference in fibre type proportions between the superficial and deep regions, with more type 1 fibres in deep than in superficial regions and more type 2a and 2b fibres at the surface than in deeper regions. Therefore, in VML and VMO, the site of biopsy should be well defined. It is not clear whether in humans such a distribution reflects current functional demands placed on VM, or if it is just a vestige of an ordered spatial distribution, possibly needed to facilitate survival, still clearly present in some animal muscles. Differentiation of muscle fibres into distinct types originates early in development. The pattern of muscle fibre types progressively evolves from a homogeneous primordium, as a consequence of myotubes, differentiating according to the time of their formation and their position within the muscle. In small rodents two generations of myotubes are formed by temporally discrete phases of myoblast fusion (Rubinstein & Kelly, 1981; Harris et al. 1989; Condon et al. 1990). A similar temporal course of differentiation was noticed in birds (McLennan, 1983), whereas in larger mammals such as sheep (Wilson et al. 1992) and man (Draeger et al. 1987), three generations of myotubes were observed. In addition to the time of formation, the fate of muscle cells is also determined by their position within a muscle (Harris et al. 1989; Condon et al. 1990). In many rat hindlimb muscles, slow muscle fibres are concentrated in deeper, axial portions, whereas fast fibres predominantly occupy the superficial portion. The result of such a temporal and spatial regulation of fibres is that, at birth, muscles contain a mixture of fibre types and that within particular muscles fibre types are not randomly intermingled in a mosaic pattern, but have an ordered spatial distribution. The differentiation of muscle fibres continues in postnatal life, and as the animal or human matures, the fundamental plan is modulated by environmental effects. Nevertheless, there is some evidence that adult animal skeletal muscles retain the spatial pattern, introduced early in muscle histogenesis (Armstrong et al. 1982; Armstrong & Phelps, 1984; Lexell et al. 1994). As far as our knowledge is concerned, it is not clear whether human skeletal muscle fibres undergo a similar spatial pattern of differentiation. However, analyses of adult vastus lateralis muscle, which is one of the most studied human muscles, gave conflicting results. Evidence supporting the possibility of regional patterns of fibre specialisation was provided by Johnson et al. (1973) and Lexell et al. (1983), who found a predominance of type 2 fibres at the surface and type 1 fibres in deep regions. Others, however, have found a nonsystematic difference in the distribution of fibre types (Elder et al. 1982; Nygaard & Sanchez 1982; Mahon et al. 1984; Eržen et al. 1990).

In conclusion, results of this study provide evidence that the VML and VMO are not only anatomically and functionally different muscles, but that their histochemical characteristics are also significantly different. Differences in fibre type sizes are evident as well, but are less pronounced. A similar function and fibre type constitution of the VL and VMO might in part explain the difficulties in selectively strengthening the VMO muscle (Møller et al. 1987; Hanten & Schulthies, 1990; Grabiner et al. 1991).

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